ABSTRACT

TITLE OF THESIS: DEVELOPMENT OF AUDITORY SENSITIVITY IN BARN OWLS (*Tyto furcata pratincola*)

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The development of hearing in the juvenile barn owl was investigated using tones (500 Hz to 12 kHz) and clicks of different rates (5-90 Hz). Auditory evoked potentials (AEPs) of the auditory nerve were recorded using the auditory brainstem response (ABR). Barn owl hearing matured in a similar trend to other developing vertebrates, including kittens, budgerigars, chickens, and gerbils. The onset of hearing began sometime earlier than the second week post-hatch, and proceeded in a frequency-dependent manner. Adult-like thresholds were reached in a progression from low to high frequency, and sensitivity was mature by P60. These patterns were consistent with CAPs recorded from juvenile barn owls in Europe. ABRs for clicks presented at 5-60 Hz demonstrated increasing amplitudes and decreasing latencies as barn owl chicks aged, while ABRs for clicks presented at 90 Hz were barely distinguishable between adults and juveniles.

DEVELOPMENT OF AUDITORY SENSITIVITY IN BARN OWLS

(*Tyto furcata pratincola*)

by

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DEDICATION

This work is dedicated to all of the barn owls that I have had the privilege of studying over the past several years. Thank you.

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INTRODUCTION

The barn owl (*Tyto alba*) is highly-adapted as a nocturnal predator that can hunt in complete darkness, relying solely on auditory cues (Payne, 1971; Knudsen, *et al.*, 1979). It has several auditory specializations that allow it to localize sounds in space with great precision (1-5° in azimuth and elevation), utilizing a narrow range of frequencies between 5-9 kHz (Payne, 1971; Konishi, 1973 a & b; Knudsen *et al.*, 1979; Coles & Guppy, 1988). The barn owl has an acoustically-opaque facial ruff that funnels sound into the ear canals and boosts behaviorally-relevant frequencies (Coles & Guppy, 1988). The greatest amplitude gain from the facial ruff occurs between 5-8 kHz. Moreover, the barn owl has asymmetrical ear canals that allow it to utilize the difference in the sound level arriving at each ear, or the interaural level difference (ILD), to localize sounds in the vertical plane (Norberg, 1977; Knudsen, 1980; Konishi, 1983). Its basilar papilla is much longer than most other bird species. This allows the barn owl to hear a wider range of frequencies and includes an "auditory fovea", where more space and neurons are devoted to coding each frequency than in other bird species, centered around 6-10 kHz (Köppl *et al.*, 1993). The barn owl can also utilize small differences, on the order of microseconds, in the time of arrival of a sound to each ear (interaural timing difference, or ITD) to localize sounds on the horizontal plane (Knudsen, 1980; Konishi, 1983; Sullivan & Konishi, 1984; Köppl, 1997; Carr & MacLeod, 2010), due to the ability of neurons in Nucleus Magnocellularis (NM) to phase-lock up to 10 kHz. The time of arrival of sounds to each ear is coded in NM and projected via delay lines to Nucleus Laminaris (NL)—a collection of coincidence detector neurons that compute the ITD by comparing input from each ear (Jeffress, 1948; Konishi and Carr, 1990). The Inferior

Colliculus (IC) integrates the ITD and ILD information, creating a topographic map of auditory space that the barn owls use to localize sounds (Knudsen & Konishi, 1978). These auditory specializations not only make for an effective nocturnal predator but also have established the barn owl as a model for the study of audition, particularly sound localization (Konishi, 1999). As such, many aspects of the barn owl auditory system have been carefully studied.

Barn owls are altricial birds that accomplish most of their growth after birth; many features of their anatomy and physiology, including their sense organs, are immature upon hatching. Unlike precocial birds, such as chickens, that hatch with fully-functional sensory systems (Kubke & Carr, 2000), significant morphological changes occur over the course of post-hatch development in barn owls, and their hearing range, sensitivity, and frequency tuning are not adult-like for some time (Haresign & Moiseff, 1988; Köppl & Nickel, 2007). Many changes occur over the first two months. On the periphery of the auditory system, the diameter of the head doubles, the facial ruff grows in, and the ear canals increase in size. Neural development includes myelination of the delay lines from NM to NL, and changes in the size of NM neurons, the size of their endbulbs, and the length and number of their dendrites (Haresign & Moiseff, 1988; Carr & Boudreau, 1996; Cheng & Carr, 2007; for review, see Kubke & Carr, 2000).

Thus, the two months post-hatch are a critical period in the development of the barn owl auditory system; however, to date only one study has been published following the maturation of hearing in juvenile barn owls (Köppl & Nickel, 2007). This study was performed in a closed field, recording from the round window of the cochlea—an experimental preparation that did not allow the researchers to follow the development of

auditory sensitivity within the same animal over time. The current study used the auditory brainstem response (ABR), a less-invasive, non-terminal method that allows the study of hearing within the same animal over the course of development. The ABR has been used as a tool to study the development of auditory sensitivity in a wide variety of vertebrates, including cats, gerbils, budgerigars, canaries, and chickens (Saunders *et al.*, 1973; Burkard & Voigt, 1989; Burkard *et al.*, 1996a,b; Walsh *et al.*, 1996a,b,c; Brittan-Powell *et al.*, 2002a,b; Brittan-Powell & Dooling, 2004), but to our knowledge, there exists only one other study of ABRs in strigiforms (Brittan-Powell & Lohr, 2005). There are no ABR studies examining the development of hearing in juvenile owls.

The purposes of this study were 1) to examine the normal development of hearing range, frequency tuning, sensitivity, and neural adaptation of the barn owl chick between two weeks and two months post-hatch, and 2) to determine when these parameters become adult-like.

METHODS

Subjects

Nine barn owls were subjects in this experiment, two adults and seven juveniles. All subjects were housed in the Central Animal Resources Facility (CARF) at the University of Maryland, College Park on a twelve-hour light/dark cycle, with free access to food, under IACUC Protocol 417076-3. The two adult barn owls included in this study were kept in flight cages within the owl colony. The barn owl chicks were taken from two different broods produced by the same parents in the breeding colony over the course of three months. The chicks were fed several times a day by the experimenter, and kept together in a heated, humidified incubator until they all reached at least one-month of age, when they were moved to a larger cage.

The ages of the chicks were determined using estimated hatch date, weight, and head width (Haresign & Moiseff, 1988; Köppl *et al.*, 2005). To determine whether the barn owl chicks used in this study grew normally, mass and head width measurements were taken from the January clutch of chicks, compiled, plotted, and compared with growth data from other studies. **Figure 1: Owl Chick Growth** displays plots of head width and mass over time. Studies of North American barn owls (*Tyto furcata pratincola*) report rapid growth of head width between P10-30, tapering into a plateau at P35 around 45 mm (Haresign & Moiseff, 1988; Carr & Boudreau, 1996). The January chick measurements are consistent with these data: head width increased rapidly until P30, and plateaued near P35 at 45-50 mm (Figure 1 A) Head Width). Hand-raised owlets are known to lag somewhat behind nest-raised owlets in growth, but the chicks used here

routinely fell into the mass ranges described in previous studies (Haresign & Moiseff, 1988; Köppl *et al.,* 2005). Haresign and Moiseff report a rapid increase in mass between P10-40, which overshoots and then plateaus at 475 g around P50. This trend is also reflected in the measurements for the January clutch—mass increased rapidly until after P40, shot above 600 g, and settled around 500g after P50 (Figure 1 B: Body Mass).

ABRs were recorded from the juveniles as frequently as every 2-3 days over the course of two months, beginning after two weeks of age and ending after fledging, when ear canal and facial ruff growth had ceased (**Table 1: Data Collection**). No bird was subjected to recordings on consecutive days. Reference data from the two adult barn owls were obtained in a single recording session for each bird.

Anesthesia

To minimize motion artifacts, the owls were sedated prior to each experiment via an injection of ketamine (22 mg/kg) and diazepam (5.6 mg/kg). Older chicks and adults received an intramuscular injection (IM) while young chicks received a subcutaneous injection (insufficient muscle mass to administer the injection IM). Each owl was wrapped in a towel and placed on a heating pad that was maintained at 38˚C for the duration of the recording and recovery. Most of the barn owl chicks remained motionless for at least 80 minutes while some of the older chicks and adults remained anesthetized for 90-100 minutes. An additional injection (half of the initial dose of ketamine and diazepam) was given if the bird woke up before testing was complete, but this happened very rarely. The owl was allowed to recover on the heating pad until it was awake and responsive, at which point it was returned to the incubator or flight cage.

Procedure

For each experiment, the owl was anesthetized and placed onto a heating pad, positioned such that its right ear was 30cm away from the speaker (Orb Audio Mod1x, 80 Hz-20kHz, Orb Audio LLC, New York, NY). Electrodes (platinum alloy, Grass F-E2, West Warwick, RI) were placed subcutaneously behind the left ear canal (ground), at the vertex (active), and behind the right ear canal (reference). Recording electrodes fed into a Medusa Digital Biological Ampifier system (RA4L Headstage and RA16PA PreAmp; RA16BA Medusa Base station). The Medusa headstage added an additional 10x gain to the signal.

Stimulus presentation, ABR acquisition, equipment control, and data management were coordinated using a TDT System 3 modular rack-mount system (Tucker-Davis Technologies, Gainesville, FL), controlled by a computer (2.66 GHz Pentium-4 PC, TDT P15 Gigabit interface PCI card, TDT BIOSIG software). Stimuli were generated in SIGGEN (TDT software), fed through a RP2.1, and passed through a TDT PA5 programmable attenuator directly to the speaker. Stimulus intensities were calibrated using a sound level meter (System 824, Larson Davis Inc, Provo, UT) to measure continuous tones 30 cm from the speaker at the location of the bird's ear, using the fast-weighting A-scale (dB SPL). The intensity of the click was determined from its peak equivalent SPL. The voltage output of the click was compared to the voltage of a 1kHz tone, and the SPL of the tone required to match the voltage output of the click was determined to be the peak equivalent SPL of the click.

Stimuli

This study was designed to follow the development of frequency coding for the most sensitive and behaviorally relevant portion of the barn owl hearing range. The frequencies tested include 500 Hz and 1, 2, 4, 6.3, 8, 10, and 12 kHz (after Dyson *et al.*, 1998). Stimuli were arranged as multiple-intensity stimulus trains, in which different tones were presented in a series of 9 increasing sound pressure levels, at a rate of 4/s. Each tone was 5 ms long with an interstimulus interval of 20 ms. Clicks were of 0.1 ms duration, presented at 90 dB SPL with a varying interstimulus interval (see below) (Brittan-Powell *et al.*, 2002; Brittan-Powell & Dooling, 2004; Brittan-Powell *et al.*, 2005).

The intensities at which the tones were presented depended on the age and threshold range of the barn owl chick. Generally, four or five different stimulus trains were used, in addition to the five click trains. When the chicks were less than one-month old, a collection of every tone was presented in increasing steps of 5 dB from 45 or 55 dB to 85 or 95 dB, respectively. As the thresholds became more disparate, tones were presented in series of proximal intensities beginning between 0-25dB and going up to 55-80dB. For the rate experiment, click trains were presented at different rates: 5 Hz, 10 Hz, 30 Hz, 60 Hz, and 90 Hz at a constant intensity (90 dB).

An average of 300 presentations of the stimulus train, alternating phase to eliminate the cochlear microphonic, produced a single ABR trace. The recording period began at the onset of the stimulus and continued for 235ms (20 kHz sampling rate). The signal was amplified (x 100K) and notch filtered at 60 Hz. Each stimulus train was

presented twice to produce replicates at all intensity levels. The traces were filtered (offline in BIOSIG) between 30-3000Hz.

Analysis

ABR thresholds were determined for each frequency by the visual detection method, in which the first peak of the ABR (Wave 1) was followed across traces, from highest SPL to lowest, until the response was no longer visible (Brittan-Powell & Dooling, 2004; Brittan-Powell *et al.*, 2005). Threshold was defined as halfway between the SPL where Wave 1 was last detected and the SPL where it was no longer present, for e.g., if wave 1 were last detected at 20 dB, and not visible at 15 dB, the threshold would be recorded as 17.5 dB. To plot the data, thresholds were averaged between birds across 3 day periods, except for the last two periods, which were 5 day ranges. Every period included data from at least two different chicks, except for P13-15, which comprises data from two subsequent recordings from one chick. P0 was designated as the day of hatch. For a complete description of which recordings contribute to each period, as well as an outline of the age ranges used (**Table 1: Data Collection**). The recordings from the two adult barn owls (aged 5 and 9 months) were also averaged together to generate the adult reference threshold.

To determine whether the barn owl chick thresholds differed significantly from adult thresholds, a one-way ANOVA was performed within each frequency on the individual data for each owl (MATLAB, r2012a, MathWorks, Natick, MA). Post-hoc analysis (MATLAB multiple comparison test) specified which age ranges had thresholds that differed significantly from the adult thresholds. P-values were Bonferroni corrected

for the multiple tests. For the juvenile owls, adult threshold was defined as the first age-range that did not differ significantly from the adult audiogram. The results of these ANOVAs are described in **Table 2: Frequency ANOVAs**.

For the rate experiment, the amplitude and latency of the first wave were compared across each click presentation rate. The amplitude of the baseline voltage and the peak of the first wave were measured in BIOSIG and exported to Excel (Microsoft Office 2010, Microsoft, Redmond, WA). **Figure 2: ABR Waveform** gives an example of an ABR with labels denoting the features of the waveform that were used to calculate amplitude and latency, including the peak of the first wave and the baseline. In order to calculate the peak amplitude, the baseline voltage was subtracted from the peak voltage of the first wave. To calculate latency, the time-point of the first wave was measured in BIOSIG, at the peak of the wave, and exported to Excel. The amount of time it took after stimulus onset for the sound to travel 30 cm from the speaker to the owl's ear (0.88 ms) was subtracted from the time-point of the first wave peak to obtain the latency of signal conduction from the ear canal to the auditory nerve. Peak latencies and amplitudes were averaged between chicks across 3 day periods (again, except for the last two periods, which were 5 day ranges), and between the two adults.

To determine when barn owl chick amplitudes and latencies became mature, a one-way ANOVA was performed within each click rate on the individual data for each owl (MATLAB). Post-hoc analyses (MATLAB multiple comparison test) specified which age ranges had amplitudes and latencies that differed significantly from the adult thresholds. P-values were Bonferroni corrected. Adult-like amplitudes and latencies

were reached at the first juvenile age-range that did not differ significantly from the adults. The results of these ANOVAs are reported in **Table 3: Click Rate ANOVAs**.

RESULTS

ABR Waveform Morphology

Changes in the morphology of the ABR waveform gave insight into the development of the barn owl auditory system. A typical ABR trace has a major peak, Wave 1, followed by several additional peaks. For the purposes of this study, we have focused on Wave 1. The shape of ABR Wave 1 changed in four major aspects as the barn owl chicks aged: threshold, amplitude (magnitude of the peak), sharpness (peak width), and latency (time to peak). A diagram of these features can be found in **Figure 2: ABR Waveform**. The amplitude, sharpness, and latency of Wave 1 were assessed qualitatively for tones. For clicks, the amplitude and latency of Wave 1 were quantified to determine the effects of click rate on neural synchrony, adaptation, and fatigue (see: **ABRs for Clicks**). Thresholds were determined for each tone using visual detection (see: **ABR Thresholds by Frequency**).

Within a constant frequency and SPL, the latency of the response decreased as the chicks matured, while the amplitude and sharpness of the peak increased over time. In other words, when response to a certain frequency first emerged, the latency of response was longer, the width of the peak greater, and the amplitude of the peak smaller than when the chick aged. Adult-like responses had a shorter latency, and narrower and larger peaks for the same stimulus (**Figure 3: ABR Waveform Over Time**). For owls of all ages within one experiment, as sound level decreased within a constant frequency, the latency of the ABR increased, and the peak amplitude and width decreased.

ABR Onset by Frequency

The chicks used in this study were first tested around 2 weeks of age, prior to which barn owl chicks been described as being functionally deaf, with cochlear action potentials visible only at very high SPLs, generally not found in nature (Koppl &and Nickel, 2007). P13 was the earliest age that any barn owl chick was tested, but most chicks were tested beginning at P16. Onset was defined as the age at which the majority of barn owl chicks exhibited a response to a given frequency, at intensities below 80 dB SPL (see **METHODS**). The onset of hearing progressed from low to high over the range of frequencies tested, with the exception of 500 Hz, which had an onset slightly later than 1-5 kHz. 500 Hz was the lowest frequency tested.

Between P13-15, juvenile barn owls exhibited responses to frequencies between 1-5 kHz at amplitudes below 80 dB SPL. No chicks responded to frequencies above 5 kHz at ages between P16-18. Some, though not all, also responded to 500 Hz at P16-18, and all chicks responded to 500 Hz by P19-21. The onset of responses to 6.3 kHz occurred at P19-21, for 8 kHz about a week later (P25-27), and 10 kHz a week after that (P31-33). Finally, onset for 12 kHz was recorded at P40-42.

These changes in frequency range can be observed in the audiograms presented in **Figure 4: Audiograms by Age**. For the earliest audiograms (P13-15 and P16-18; Figure 4 A)), the plot flattens out into a plateau at 80 dB above 5 kHz, indicating that, on average, birds of that age did not exhibit ABRs to those frequencies at an intensity below 80 dB. The threshold of the chicks' responses for tones above 5 kHz began to drop below the 80 dB plateau as they matured, in a sequence from low to high frequency (6.3-

12 kHz). Both graphs demonstrate that for 12 kHz, even after response onset at P40-42 (Figure 4 B)), the threshold for this tone did not drop but remained near 70 dB SPL (thresholds will be discussed in more detail below). Thus, the onsets of all frequencies tested here were found to be complete at P40-42, after the doubling of barn owl chick head size but prior to the full growth of the facial ruff (Haresign & Moiseff, 1988).

ABR Thresholds by Frequency

Thresholds for each frequency were determined by visual detection (as described in **METHODS**), which has been demonstrated to be more accurate at determining threshold than automated algorithms (Brittan-Powell & Dooling, 2004; Brittan-Powell *et al.*, 2005). Barn owl chicks' thresholds were recorded for each frequency during every ABR experiment, binned according to age, and averaged within each bin. The audiograms derived from these average thresholds are displayed in **Figure 4: Audiograms by Age**. After the onset of response in each frequency tested (see above: **ABR Onset by Frequency**), the shape of the audiogram remained relatively constant, while the position on the y-axis shifted to lower intensities as the barn owl chicks aged. The most sensitive portion of the audiogram is between 2-8 kHz. The shape of the audiograms reported here are consistent with other audiograms recorded in the barn owl (Dyson *et al.*, 1998; Koppl & Nickel, 2007; Koppl & Gleich, 2007).

In order to determine when the barn owl chicks' thresholds became adult-like, one-way ANOVAs were performed on the raw threshold data for each frequency. P-values were Bonferroni corrected and used to determine the point at which the juveniles' thresholds were statistically indistinguishable from the adults' thresholds for each

frequency. The results of the ANOVAs are recorded in **Table 2: Frequency ANOVAs**. The barn owl chicks' thresholds for each tone decreased and approached the adult reference threshold over time, as described above; however, the age at which adult-like thresholds were reached varied with frequency. **Figures 5-7: Thresholds by Frequency** track the change in threshold for each frequency across age. In general, responses to lower frequencies matured earlier than higher frequencies, with some exceptions. For example, 6.3 kHz reached adult threshold at P22-24, about 20 days earlier than proximal frequencies. 500 Hz through 8 kHz were adult-like by P45, but 10 kHz took another week (P49-51), and 12 kHz was not adult-like until the very end of the recording period (P57-61).

ABRs for Clicks

In order to characterize the capacity for adaptation of the juvenile barn owl, we measured responses to clicks presented at different rates. Five different click rates were tested: 5 Hz, 10 Hz, 30 Hz, 60 Hz, and 90 Hz. The amplitude of the first wave of the ABR was measured as the difference between the peak of the wave and baseline (see **Figure 2: ABR Waveform**). Latency was measured as the amount of time between the sound reaching the barn owl's ear and the peak of the first wave. The amplitudes and latencies of the ABRs for each click rate were divided into age groups, averaged, and plotted in **Figure 8: Click Amplitude and Latency**. For a constant age, the amplitude and sharpness of the ABRs decreased, while the latencies increased, as click repetition rate rose. Thus, ABRs to 5 Hz exhibited a large, sharp peak and a short latency, while ABRs to 90 Hz had low, broad peaks and a longer latency. As the chicks aged, responses for clicks exhibited a similar pattern as the morphological changes observed for tonal

stimuli: over time, the amplitude and sharpness increased within each click rate, and the latencies decreased (**Figure 3: ABR Waveform Over Time**). For example, ABRs for 5 Hz recorded for a chick at P16 had smaller, wider peaks and longer latencies than ABRs recorded for the same chick and stimulus at P40.

Statistical analyses were used to determine when the amplitudes and latencies of the barn owl chicks' click responses became adult-like. One-way ANOVAs were implemented for each of the five click rates, post-hoc tests performed, and the results Bonferroni corrected to compensate for multiple tests. The results of these ANOVAs are recorded in **Table 3: Click Rate ANOVAs**. Post-hoc tests revealed that the amplitudes of the barn owl chick responses for click rates from 5 to 60 Hz were not mature for any of the ages tested—in other words, that the amplitudes of the adult click responses were significantly different from the amplitudes of the barn owl chicks, even for juveniles aged up to P61. For 90 Hz, adults did not differ significantly from the barn owl chicks. 90 Hz was the highest click rate presented, and the ABRs recorded for 90 Hz always had the lowest, widest peaks with longest latencies for both the adults and juveniles (see previous paragraph).

In contrast to the ABR amplitudes, the ABR latencies for click rates matured in a rate-dependent fashion. Post-hoc tests revealed that barn owl chicks' latencies were different from adults' until P19-21 for 5 to 30 Hz. For 60 Hz, chicks' latencies became adult-like at P28-30. Responses for 90 Hz from adults and chicks differed only for the youngest group, indicating that latencies were adult-like at P16-18.

DISCUSSION

Summary of Findings

This study has characterized the normal development and maturation of barn owl hearing. Several properties of the barn owl auditory system were examined, including hearing range, frequency tuning, sensitivity, and neural adaptation, by measuring the auditory brainstem response (ABR) between two weeks and two months post-hatch.

The maturation of Wave 1 of the ABR waveform progressed in a manner similar to that of other developing vertebrates: For the same stimulus, the amplitude and sharpness of the peak increased, and the latency of the response decreased as the chicks aged (Brittan-Powell & Dooling, 2004; Burkard *et al.*, 1996a). Most notably, this pattern of maturation was consistent with the other study in juvenile barn owls (Köppl & Nickel, 2007). For owls of every age, during one experiment day, decreasing the presentation intensity of a tone resulted in a smaller amplitude, wider peak, and longer latency of Wave 1. This pattern was also observed in other vertebrates, including budgerigars, kittens, and gerbils (Brittan-Powell & Dooling, 2004; Burkard *et al.*, 1996a; Burkard & Voigt, 1989). A discussion of the similarities in hearing development between barn owls and other bird species is located in **Comparison to Other Birds.**

The onset of hearing for all frequencies tested (500 Hz, 1-12 kHz) was found to be complete by P40-42. Morphologically, at this age the barn owl head had doubled in size and the post-hatching growth of the ear drum, also indicative of middle-ear growth, had ceased, but the facial ruff was not yet fully developed (Haresign & Moiseff, 1988; Köppl *et al.*, 2005). Hearing onset had already occurred by P16 for 1-5 kHz. Thus, no conclusion could be made from these data as to the order in which these frequencies

became audible to the barn owl chick, but results were comparable to the barn owl chick study by Köppl & Nickel (see **Comparison of CAPs and ABRs**).

Thresholds for tones attained adult-like sensitivity in a gross pattern of maturation from low to high frequency. This progression is consistent with the course of apical to basal maturation in the basilar papilla (Köppl & Nickel, 2007). However, there were a few exceptions. Most notably, 6.3 kHz matured early (P22-24) compared to proximal frequencies (P40-45). This result was unexpected, and will be discussed further in a subsequent section. Overall, thresholds for 500 Hz through 8 kHz were adult-like by P45. Responses to 10 kHz matured by P49-51, and 12 kHz at P57-61. Therefore, barn owl frequency tuning was observed to be adult-like near P40, but adult-like sensitivity was delayed: Thresholds continued to mature for about more 3 weeks, reaching maturity near P60. An examination of the similarities and differences between the results reported here and the findings of Köppl and Nickel can be found in **Comparison of CAPs and ABRs**.

Wave 1 amplitudes and latencies from click rate trials were quantified and analyzed, and matured over two distinct time-courses. Clicks were presented at 90 dB at different rates: 5, 10, 30, 60, and 90 Hz. For clicks presented at rates between 5-60 Hz, amplitude was not found to be adult-like for any of the ages tested between P13-P61. In contrast, for clicks presented at 90 Hz, chick amplitudes were similar to adults' at P19-21. The latencies of ABRs for clicks between 5-60 Hz matured in a sequential fashion, with lower click presentation rates becoming mature earlier than higher rates. Again, responses for clicks presented at 90 Hz were an exception—the latencies for these

responses differed from the adults only at P13-15. The implications of these findings are explored in **Responses to Click Rates**.

Comparison of CAPs and ABRs

There is one other published study of hearing development in juvenile barn owls (Köppl & Nickel, 2007). They recorded from the round window to describe changes in the cochlear microphonic and compound action potential (CAP) responses to tones (500 Hz and 1-5 kHz). These two studies utilized different methods to record changes in the potential of the auditory nerve. The correlate of the CAP is Wave 1 of the ABR; thus, we've compared our results to the CAP results described in the 2007 study.

Comparable findings were to be expected, and there were some important similarities between the results of the two studies. The 2007 study reported CAPs from barn owl chicks as young as P6. Responses at this age were restricted to 1-2 kHz, expanding to 3 kHz at P8. By P16, CAPs were recorded for 1-5 kHz. These results correspond to the ABRs recorded here at P16, which also exhibited responses between 1-5 kHz. For both CAPs and ABRs, responses to higher frequencies emerged as the barn owl chicks aged; meanwhile, thresholds for each frequency continued to decrease. CAPs reached adult sensitivity at a median age of P42 for 1-7 kHz and P65 for 8-10 kHz, while our ABRs attained adult-like thresholds at P45 for frequencies up to 8 kHz, P50 for 10 kHz, and P59 for 12 kHz. Thus, both the pattern of frequency onset and the low to high progression of frequency sensitivity maturation were alike for CAPs and ABRs.

Given that frequency onset and sensitivity progressed similarly in both studies, it follows that the shapes of the audiograms reported for CAPs and ABRs were analogous.

Köppl and Nickel report an audiogram for barn owl chicks at P60 that dropped from 50 dB SPL at 500 Hz to about 20 dB at 4 kHz. The most sensitive region of the audiogram was between 4-7 kHz, with thresholds that rose slightly for 8 kHz (near 30 dB), and jumped up to 70 dB for 10 kHz. The ABR audiogram reported here for P57-61 exhibited a similar pattern. Threshold dropped from 40 dB at 500 Hz to 17 dB at 4 kHz. For ABRs, the most sensitive region of the audiogram was 4-5 kHz. Threshold then jumped up to 25 dB for 6.3 kHz, dropped slightly to 23 dB at 8 kHz, rose to 33 dB at 10 kHz, and ended at 65 dB for 12 kHz. The sensitivity of ABRs recorded at P57-61 was adult-like for all frequencies, as reported above. Considering the findings of both studies for frequency onset, sensitivity, and hearing range, it is apparent that each method produced similar results.

There were, however, some noteworthy differences between the two studies. Köppl and Nickel described the barn owl chicks as "functionally deaf" until P14-18 because most responses occurred for SPLs 80 dB and above, at intensities greater than those normally encountered in nature (Köppl & Nickel, 2007). Our results showed responses to frequencies between 1-5 kHz for intensities below 80 dB at P13-18. This disparity persisted for frequency thresholds over the course of development, such that the ABR thresholds measured were often as much as 10 dB SPL lower than the CAP thresholds. The reason for this discrepancy is unknown, and warrants further investigation. The disparity reported here may be due to the experimental setup; Köppl & Nickel used a closed system to deliver sound stimuli, while the ABR experiments were performed in the free field. One major difference in such experimental preparations is that free field stimuli include the effects of the auditory periphery, particularly the facial

ruff and the full length of the ear canal, while closed field stimuli are delivered via headphones, and exclude these features. The external ear cavity and facial ruff provide an intensity boost to frequencies between 1-12 kHz (Coles & Guppy, 1987; Haresign & Moiseff, 1988). The ear canal begins to grow at P11, the facial ruff begins to grown in around P35, and both the ear canal and facial ruff mature around P60 (Coles & Guppy, 1987; Haresign & Moiseff, 1988). Given the time course of maturation for the ear canal and facial ruff, it is possible that the 10 dB difference could be attributed to the influence of these features of the auditory periphery.

An alternative explanation for these disparities could be a species difference. Köppl and Nickel mostly used European barn owls (*Tyto alba guttata*), while the barn owls used in this study were North American barn owls (*Tyto furcata pratincola*). Barn owl chicks of each species exhibit similar growth in head width, but *Tyto furcata pratincola* has a greater average body mass (Köppl *et al.*, 2005). It has been suggested that the many barn owl subspecies should be separated into two distinct species, as has been done for *Tyto furcata pratincola* and *Tyto alba guttata*. A recent study examined variation of the Cox1 mitochondrial gene in several barn owl subspecies, and found evidence for two clades: European and North American (Naijman & Aliabadian, 2013). More investigation is necessary to determine whether there are significant differences in hearing between European and North American barn owls.

Another noteworthy difference between the ABR and CAP studies was the threshold for 6.3 kHz. Our findings showed that this frequency reached adult-like thresholds about 20 days earlier than proximal frequencies, at odds with the low to high progression that was predominant both here and in Köppl and Nickel's study. Moreover,

a slightly-elevated threshold for 6.3 kHz, visible in the audiogram as a notch, appeared at P40 and persisted over the course of development (see **Figure 4: Audiograms by Age**). This notch was also visible in the adult reference threshold. The audiograms reported by Köppl and Nickel did not exhibit this notch, nor was there any mention of elevated thresholds for 6 kHz. However, a notch in the barn owl audiogram at 6 kHz has been reported in other studies of adult barn owls (Konishi, 1972; Dyson *et al.,* 1998; Köppl & Gleich, 2007). Thus, the discrepancy does not appear to be the presence of the notch, but rather the age at which thresholds for frequencies near 6 kHz become elevated relative to proximal frequencies. It seems unlikely that the disparity is due either to experimental methods or species, as the notch is present in the audiogram of the CAP study in adult barn owls, which was also performed in a closed field, with *Tyto alba guttata* (Köppl & Gleich, 2007).

Comparison to Other Birds

The course of development of hearing in barn owl chicks was similar to that of other juvenile birds. The budgerigar (*Melopsittacus undulatus*), a small altricial parrot, is born deaf at hatch, much like barn owl chicks (Brittan-Powell & Dooling, 2004). Budgerigars first exhibited auditory responses at P7 for low frequencies, and frequency onset progressed to higher frequencies, with threshold dropping as the chicks aged (Brittan-Powell & Dooling, 2004). This course of frequency onset and sensitivity maturation is similar to what has been described here in the barn owl chick, though budgerigars reached adult-like thresholds at 1 month, earlier than barn owls (Brittan-Powell & Dooling, 2004). The budgerigar ABR waveform matured much like the barn

owl waveform: Latency decreased while amplitude and sharpness increased as the birds aged.

Domesticated chickens (*Gallus gallus domesticus*) are a precocial species, unlike budgerigars and barn owls, and are not born deaf. Chickens exhibited adult-like thresholds to low and middle frequencies at hatching (Saunders *et al.,* 1973). Their sensitivity to high frequencies continued to improve, and adult thresholds were reached approximately 48 hours post-hatch (Saunders *et al.,* 1973). Thus, chickens demonstrated the same pattern of low to high frequency maturation that can be seen in budgerigars and barn owls, but at an earlier age in development (Saunders *et al.,* 1973). Many aspects of post-hatch barn owl auditory system development correspond to embryonic development in the chicken (Kubke & Carr, 2000). Chickens also showed increases in the amplitude of auditory evoked potentials for higher click rates as they aged, much like the barn owl chick (Saunders *et al.,* 1973). Thus, the pattern of development of barn owl chick hearing was consistent with that of other birds, though specific time-courses of hearing onset and sensitivity maturation varied with species.

Responses to Click Rates

Several aspects of the ABRs recorded for different click rates warranted further discussion. Peak amplitude increased with age, but decreased with increasing click rate, as in budgerigars and kittens (Walsh *et al.,* 1986c; Brittan-Powell & Dooling, 2004). Changes in the timing of stimulus delivery elicit a strong effect on younger vertebrates, including budgerigars, chickens, and kittens (Saunders *et al.,* 1973: Burkard *et al.,* 1996a; Brittan-Powell & Dooling; 2004). The amplitudes of the ABRs recorded here for clicks

repeated at 5-60 Hz were not found to be mature by P61. Köppl & Nickel report that CAPs took until P100 to reach adult-like amplitudes, more than a month later than P61 (Köppl & Nickel, 2007). This might suggest that properties which contribute to neural synchrony, including fiber diameter and myelination, continue to increase long after P61 into the third month post-hatch (Walsh *et al.,* 1986c; Cheng and Carr, 2007; Köppl & Nickel, 2007). Considering that the critical period for the barn owl is about 200 days, this is not entirely surprising—the brain continues to mature during this time (Bergan & Knudsen, 1993). Thus, it seems plausible that the neural architecture continues to change after the first two months of life.

In contrast, ABRs for 90 Hz clicks achieved adult-like amplitudes at P19-21, meaning that the amplitudes of ABRs for 90 Hz clicks were indistinguishable from the adults after about P20. A study of the development of hearing in kittens examined the decrease of ABR amplitude for higher click rates, and suggested that this might be due to exhaustion of the pre-synaptic pool of neurotransmitter, which would result in smaller post-synaptic potentials, fewer synchronized action potentials, and smaller ABR amplitudes for both juveniles and adults (Burkard *et al.,* 1996b). Therefore, 90 Hz may exceed the ability of the adult barn owls' auditory nerve to fire synchronously.

Latencies to clicks decreased with age, but increased with click rate. Again, this is consistent with findings in other vertebrates, including budgerigars and kittens (Burkard *et al.*, 1996a; Brittan-Powell & Dooling, 2004). The latencies of ABRs to clicks between 5-60 Hz matured in a sequential fashion, with responses to lower click rates becoming mature earlier than higher rates, and latencies for the same stimulus decreasing as the barn owl chick aged. Thus, the barn owl auditory system became more

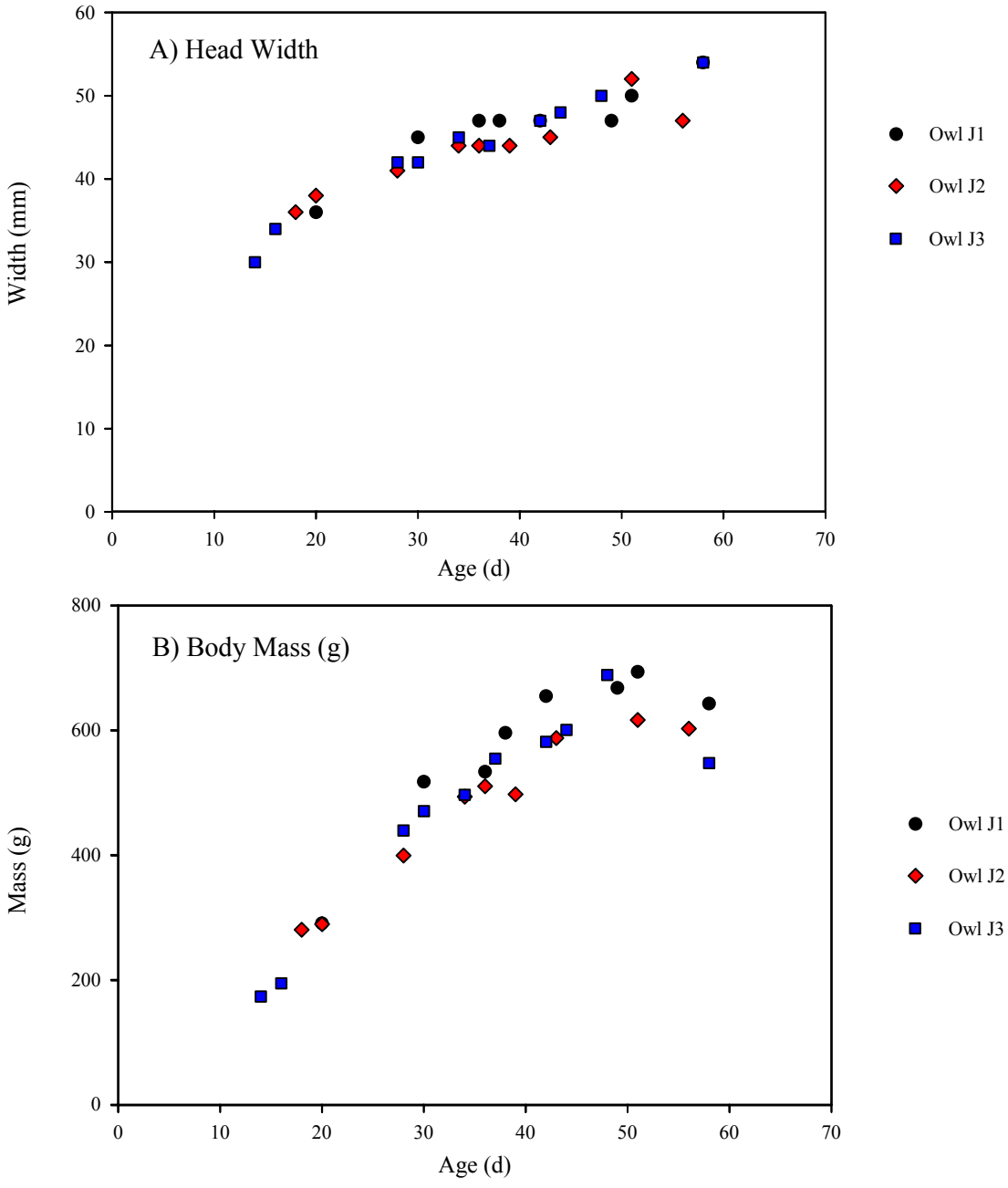
efficient at responding to lower click rates before higher ones, and efficiency improved overall as the chicks matured. Köppl and Nickel reported that CAP latencies also decreased as barn owl chicks grew, nearing adult latencies at 3-4 weeks (Köppl & Nickel, 2007). For click stimuli, decreasing latencies at P21-23 have been associated with the maturation of the endbulbs of Held, key synapses in the timing pathway between the auditory nerve and nucleus magnocellularis (Kubke & Carr, 2000). These decreases in latency with age have also been observed in other vertebrates, and have been attributed to improved mechanical transmission in the external and middle ear, as well as to faster action potential generation due to increased axon diameter, myelination, and synaptic efficiency (Katayama, 1985; Walsh *et al.*, 1986b; Brittan-Powell & Dooling, 2004). Thus, the ABR latencies reported here in barn owl chicks may depend on the maturation of neural structure and function.

The latency of ABRs recorded for click rates presented at 90 Hz differed between juvenile and adult barn owls only for the youngest group, suggesting that adult-like mechanical transmission and action potential generation were not sufficient to affect a difference in latency between the juveniles and adults. Like ABR amplitudes for clicks presented at 90 Hz, it may be that a decrease in the supply of available neurotransmitter, and subsequent smaller post-synaptic potentials, result in longer latencies at all ages (Burkard *et al.*, 1996b).

Conclusions

The development of hearing in the juvenile barn owl followed similar trends to other developing vertebrates, including kittens, budgerigars, chickens, and gerbils. The onset of hearing began sometime earlier than the second week post-hatch, and proceeded in a frequency-dependent manner. Adult-like thresholds were reached in a progression from low to high frequency, and sensitivity was mature by P60. These patterns were consistent with CAPs recorded from juvenile barn owls in Europe, though the thresholds reported here were at least 10 dB SPL lower. The reason for this discrepancy could be a difference in experimental setup or subject species. ABRs for clicks presented at 5-60 Hz demonstrated increasing amplitudes and decreasing latencies as barn owl chicks aged, while ABRs for clicks presented at 90 Hz were barely distinguishable between adults and juveniles.

Figure 1: Owl Chick Growth



Growth measurments of barn owl chicks from the January clutch.

A) Growth of head width by age. Head width increased quickly until P30, and flattened out near P35 between 45-50 mm.

B) Growth of body mass by age. Mass increased until P40, rose above 600 g, and settled around 500g after P50.

Table 1: Data Collection

Age Range (d)	Owl N1	Owl N2	Owl N3	Owl N4	Owl J1	Owl J2	Owl J3
P13-15							14, 16
P16-18	17	17	18	18			
P19-21	20	21					
P22-24	23, 25			23			
P25-27				28			28
P28-30		30	30	30	30		30
P31-33	34		34			34	34
P34-36		35			36	36	37
P37-39	40		39	38, 40	38	39	
P40-42		43	41		42	43	42
P43-45	46	45					44
P46-48					49	48	48
P49-51					51	52	
P52-56					55	56	56
P57-61					58	61	58
Total Recordings:	7	6	5	6	8	8	11

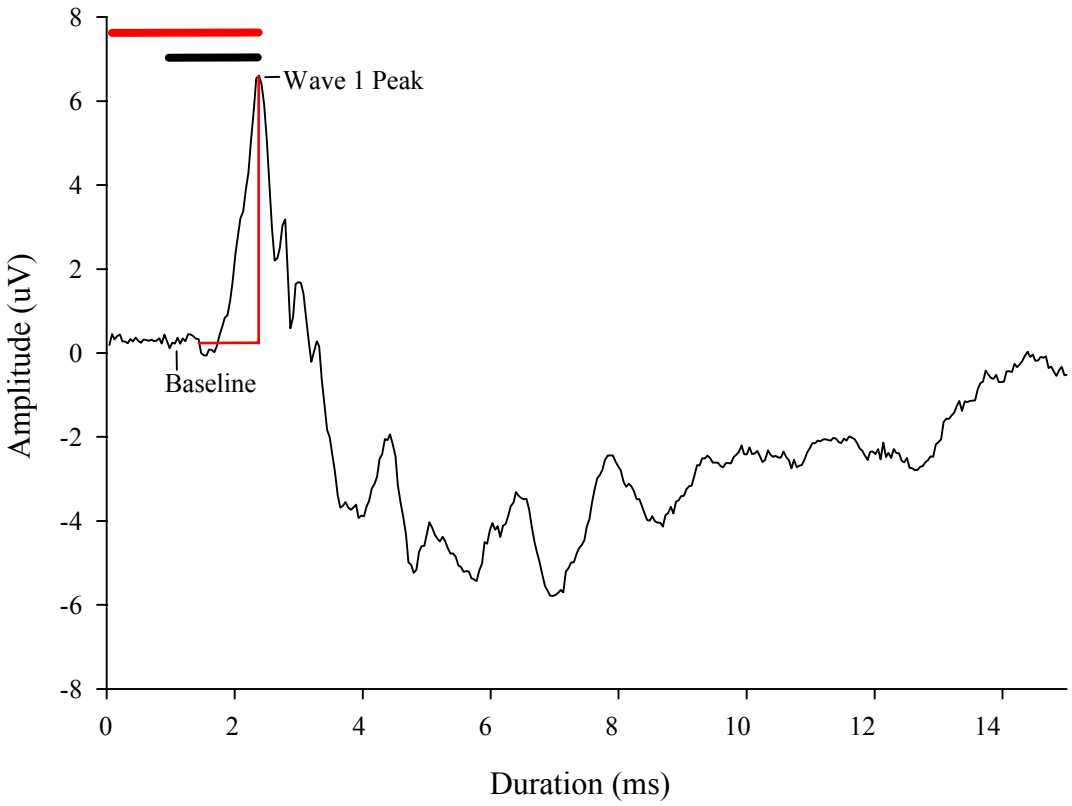
This table lists every recording day for all of the barn owl chicks used in this study, as well as the total number of recordings from each chick.

Table 2: Frequency ANOVAs

Frequency (kHz)	F value	DF	p-value	Mature
0.5	9.69	15, 96	<0.0001	P28-30
1	12.59	15, 108	<0.0001	P22-24
2	11.18	15, 109	<0.0001	P43-45
4	11.24	15, 109	<0.0001	P40-42
5	14.82	15, 108	<0.0001	P43-45
6.3	66.26	15, 98	<0.0001	P22-24
8	32.7	15, 91	<0.0001	P40-42
10	27.59	10, 66	<0.0001	P49-51
12	7.16	7, 53	<0.0001	P57-61

One-way ANOVAs and post-hoc tests were performed on the thresholds for each click rate to determine when thresholds became adult-like. P-values were Bonferroni corrected to compensate for multiple tests. Thresholds matured in a gross pattern from low to high frequency, with some exceptions.

Figure 2: ABR Waveform



This is an example of an ABR waveform. The peak of Wave 1 of the ABR is labeled, as is the baseline voltage. The baseline voltage was subtracted from the peak voltage to determine the amplitude of the peak, (indicated by the red lines). The latency of Wave 1 (fat black line) was calculated as the time from stimulus onset (0 ms) to the Wave 1 peak (fat red line) minus the amount of time it took for the sound to travel from the speaker to the owl's ear (0.88 ms).

Table 3: Click Rate ANOVAs

A) Peak Amplitude

Click Rate (Hz)	DF	F-value	p-value
5	15, 29	9.63	<0.0002
10	15, 30	7.77	<0.0002
30	15, 30	16.8	<0.0002
60	15, 30	32.13	<0.0002
90	15, 30	2.68	0.0012

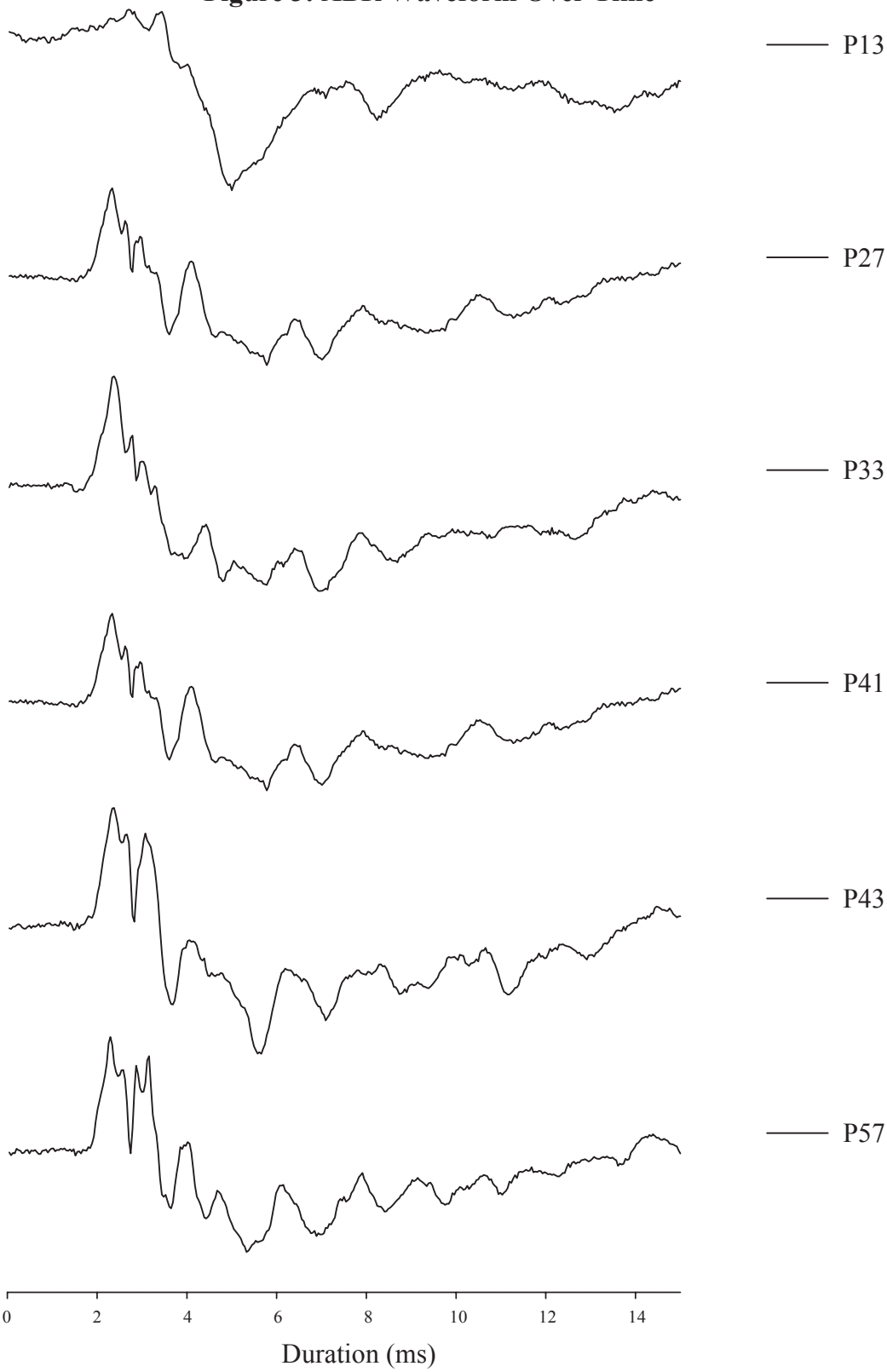
B) Peak Latency

Click Rate (Hz)	DF	F-value	p-value
5	15, 30	9.96	<0.0002
10	15, 33	8.71	<0.0002
30	15, 33	9.4	<0.0002
60	15, 33	6.69	<0.0002
90	15, 31	3.61	0.104

A) One-way ANOVAs and post-hoc tests were performed on the Wave 1 amplitudes for each click rate to determine when amplitudes became mature. P-values were Bonferroni corrected to compensate for multiple tests. 5-60 Hz never reached adult-like amplitude, but 90 Hz was mature at P19-21.

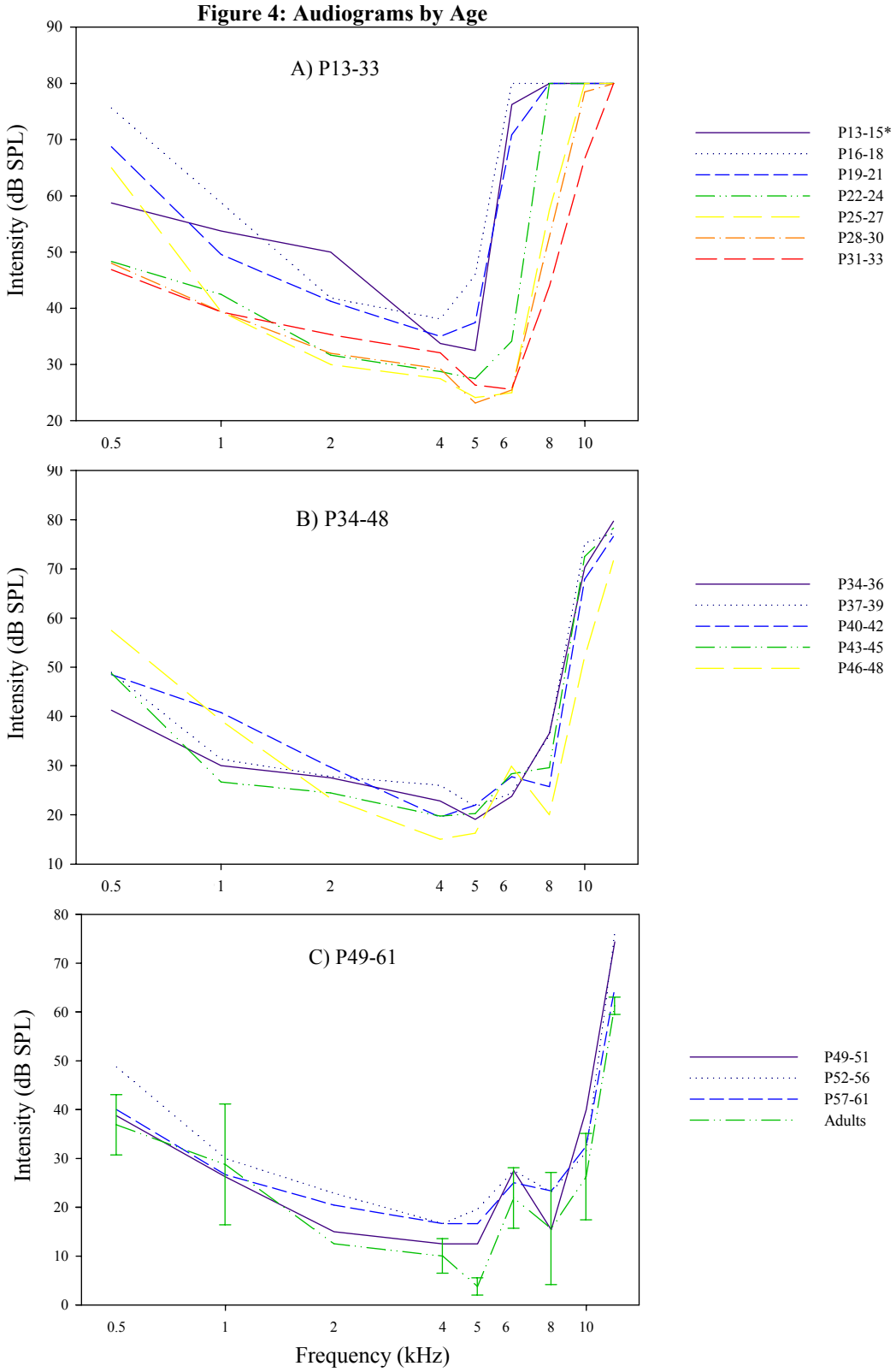
B) One-way ANOVAs and post-hoc tests were performed on the Wave 1 latencies for each click rate to determine when latencies became mature. P-values were Bonferroni corrected to compensate for multiple tests. Latencies for 5-30 Hz matured at P19-21, and 60 Hz at P28-30. Responses for 90 Hz reached adult like latencies at P16-18.

Figure 3: ABR Waveform Over Time



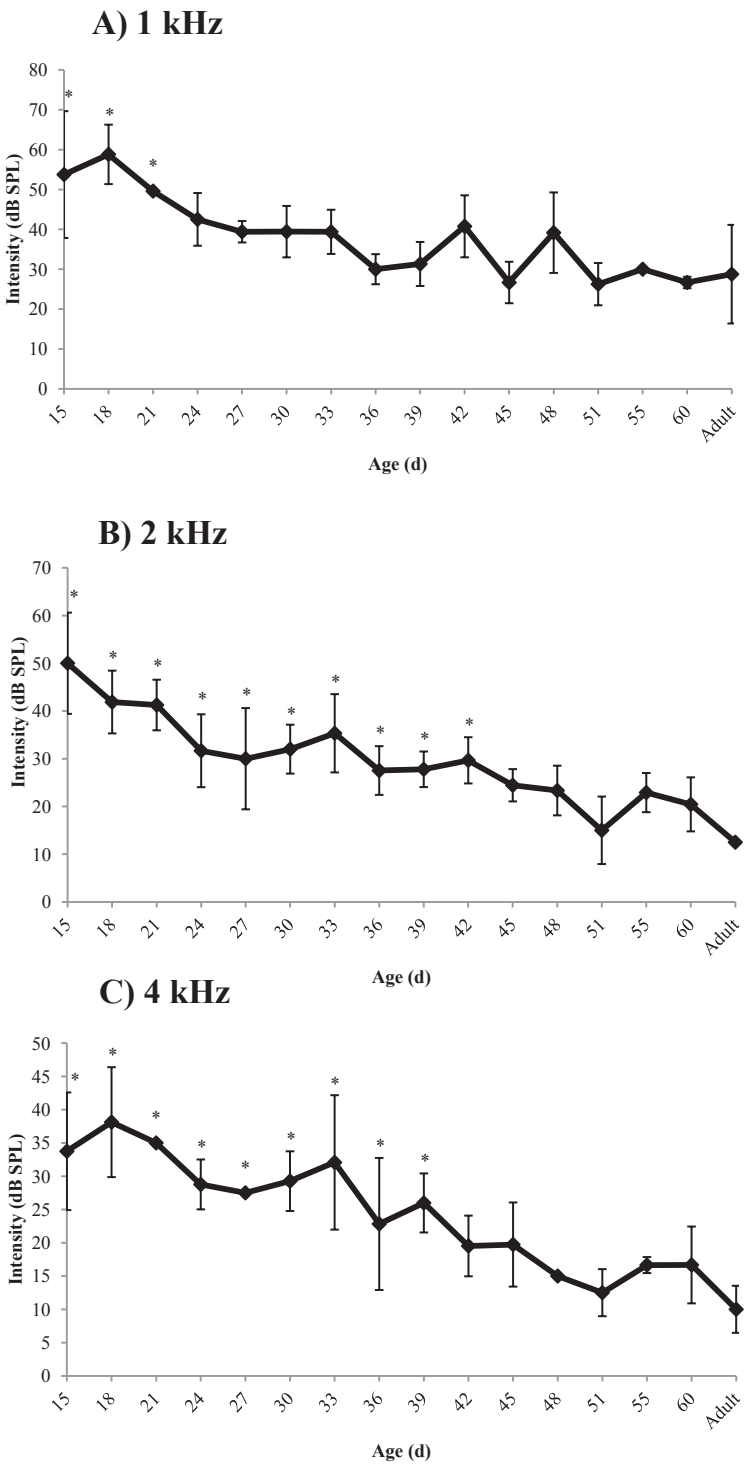
This figure shows the change in the ABR waveform for one barn owl (J3) over development (age listed on the right). The stimulus was a click repeated at 5 Hz.

Figure 4: Audiograms by Age



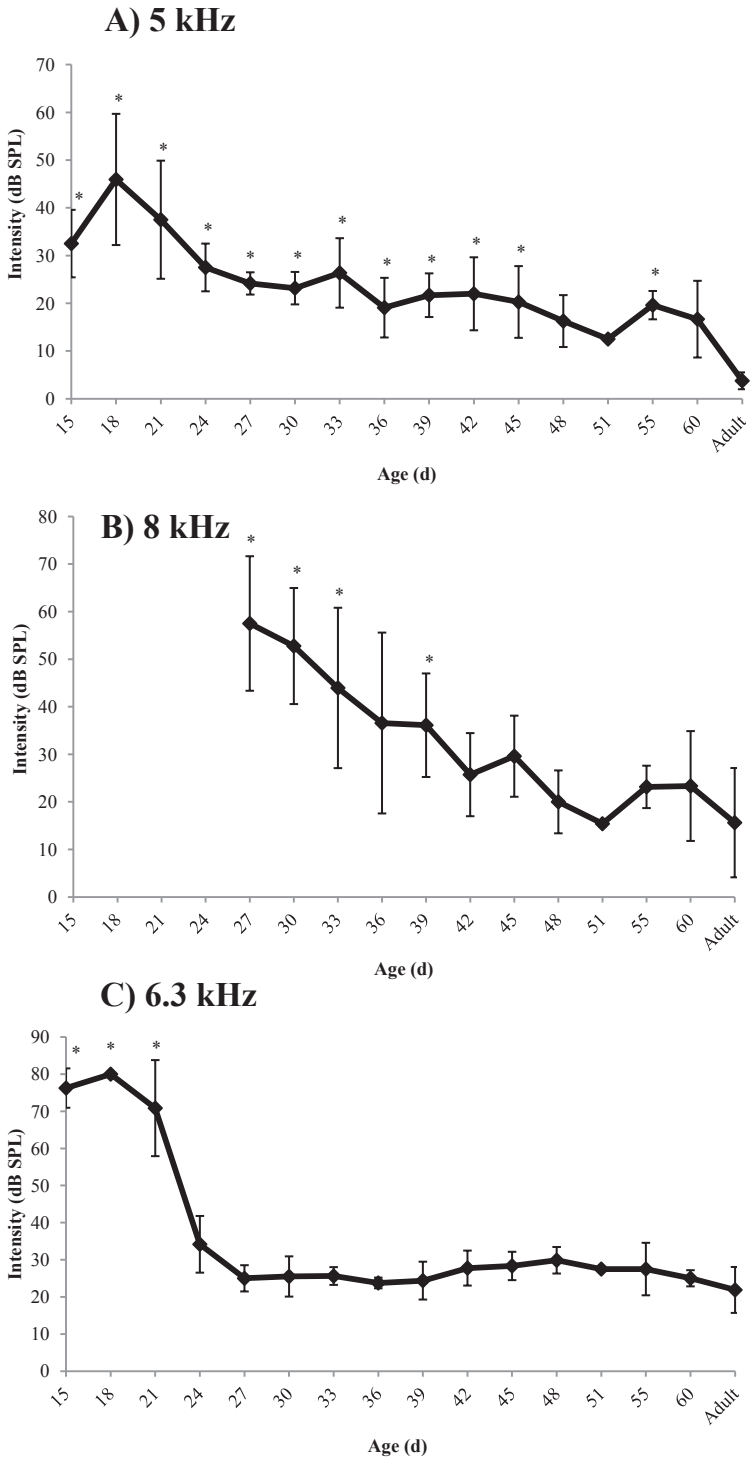
A) Audiograms for barn owl chicks, P13-33. Asterisks indicate the age group where only one barn owl was tested. B) Audiograms for barn owl chicks, P34-48. C) Audiograms for barn owl chicks, P49-61 and adult barn owls. Error bars (standard deviation) are shown for the adult reference threshold.

Figure 5: Thresholds by Frequency, 1-4 kHz



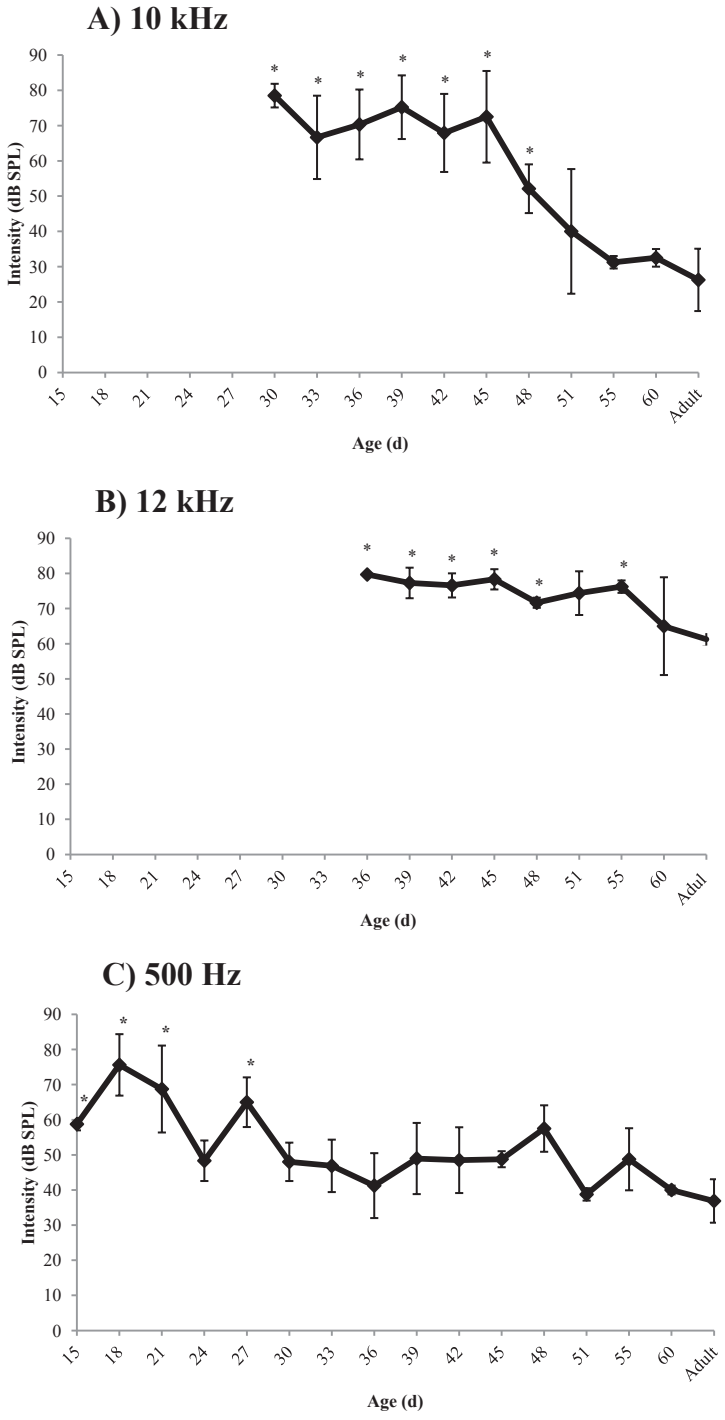
Plots A-C show threshold (dB SPL) by age (day) for frequencies between 1-4 kHz. Asterisks mark frequencies where thresholds were statistically different from adult threshold. Error bars indicate standard deviation.

Figure 6: Thresholds by Frequency, 5-8 kHz



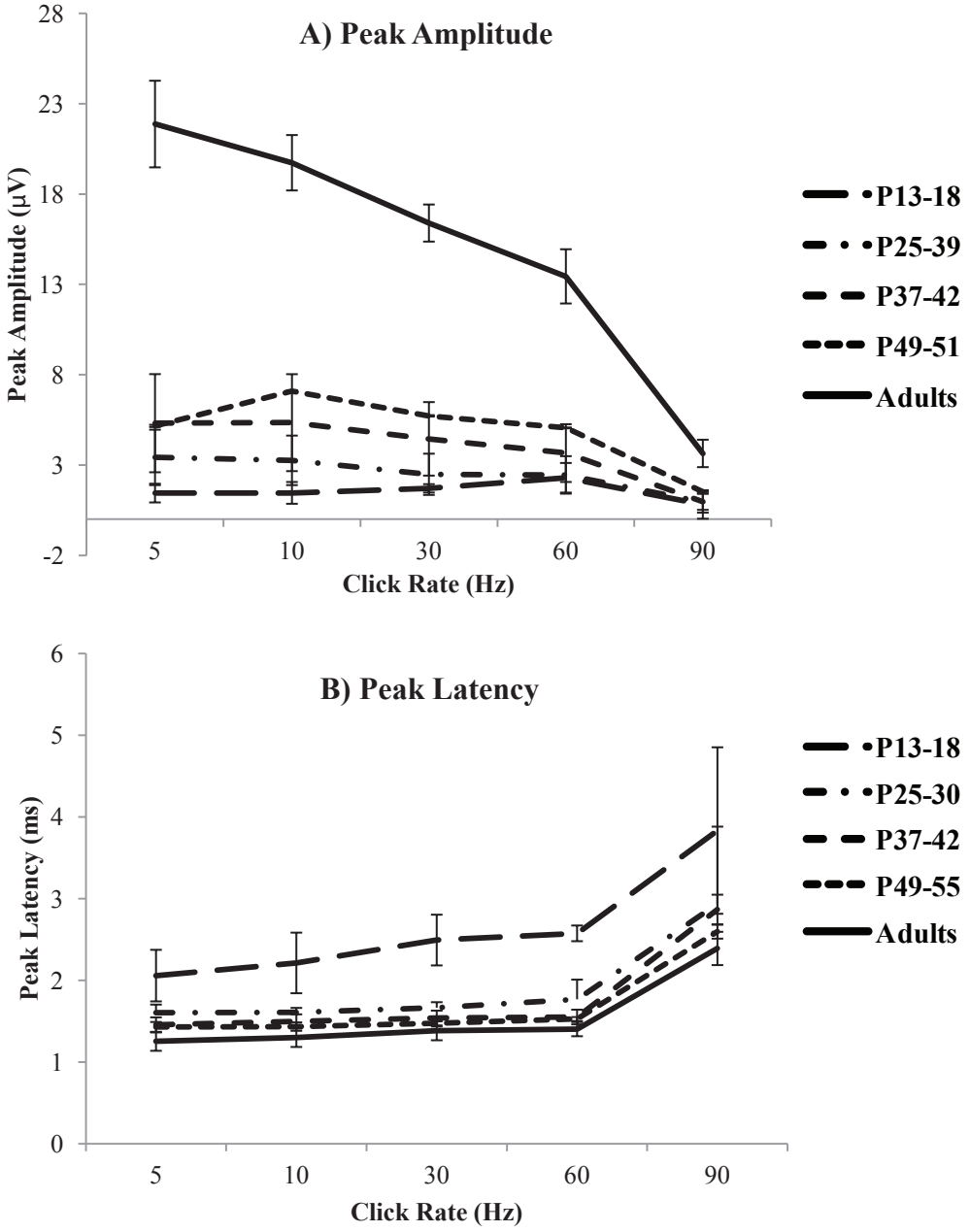
Plots A-C show threshold (dB SPL) by age (day) for frequencies between 5-8 kHz. Asterisks mark frequencies where thresholds were statistically different from adult threshold. Error bars indicate standard deviation.

Figure 6: Thresholds by Frequency, Extremes



Plots A-C show threshold (dB SPL) by age (day) for different frequencies. Asterisks mark frequencies where thresholds were statistically different from adult threshold. Error bars indicate standard deviation.

Figure 8: Click Amplitude and Latency



Plot A) shows the amplitude of Wave 1 for different click rates. Several different ages are shown to illustrate the progression of amplitude as barn owl chicks age. Error bars indicate standard deviation.

Plot B) shows the latency of Wave 1 for different click rates. Several different ages are shown to illustrate the change in latency as barn owl chicks age. Again, error bars indicate standard deviation.

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